27. A recombinant human tissue factor comprising the amino acid sequence shown in Figure 2 from amino acid residue three to amino acid residue 219 expressed in a host cell selected from the group consisting of procaryotic cells, non-human animal cells, insect cells, plant cells, and yeast, having activity in a clotting assay.

28. The recombinant human tissue factor of claim 27 which is not glycosylated.

29. The recombinant human tissue factor of claim 27 comprising the amino acid sequence shown in Figure 2 from amino acid residue three to amino acid residue 263.

30. An recombinant human tissue factor fusion protein having activity in a clotting assay.

Remarks

Rejections under 35 U.S.C. §112, first paragraph

The specification has been objected to as non-enabling and claims 4-6, 8 and 20-26 have been rejected as non-enabled, under 35 U.S.C. §112. These rejections are respectfully traversed if applied to the amended claims.

Independent claims 4 and 20 have been amended to require that the tissue factor protein is human and has activity in a clotting assay. It is not the intent to claim a protein which has no biological activity. It is also not the intent to claim a protein which is not active in a clotting assay using human plasma, since tissue factor is species specific. To facilitate prosecution, the claims have been further limited to the amino acid sequence of

Figure 2, except that a cysteine can be replaced with another amino acid or an amino acid at an N- or O-glycosylation site is substituted or deleted. The cysteine-substituted form is in fact a form that is being marketed, for example, by Dade International, which is a sublicensee of this application. The Dade tissue factor is also truncated to less than the full length (approximately 243 amino acids). Therefore no inoperative species are claimed. For the benefit of the Examiner, a clotting assay literally takes seconds and has been so automated as to require almost no input by the technician. Hospitals and laboratories throughout the world conduct clotting assays - typically by adding 0.1 ml plasma plus 0.1 ml calcium chloride plus 0.1 ml phospholipid vesicles plus tissue factor (the latter in place of thromboplastin). The sample is gently agitated and the time to clot compared on a calibrated scale to determine the amount of clotting factor to be measured. An example of such a one stage assay is described on page 38 of the specification. It can be definitely determined whether or not the protein has clotting activity in an assay using human plasma; since there is so much species to species divergence, one cannot use tissue factor from non-human sources in clinical assays for human clotting factors. Accordingly, in a few seconds, one can determine if a protein meets the limitations of the claim: either it is useful in a clotting assay using human plasma or it is not.

The amino terminus of human tissue factor is naturally cleaved by post-translational processing. This information was independent ascertained and presented by Dr. Ron Bach

and Dr. James Morrissey at the Proceedings of the American Heart Association in November 1986, which cited in the Information Disclosure Statement and cited by the European Patent Office against the corresponding European application. As a result, even though the gene encodes tissue factor which includes a signal peptide and amino acids one to 263, post translational cleavage may occur prior to amino acid one (leaving one through 263), two (leaving two through 263), and three (leaving three through 263). Accordingly, the claimed protein is defined as beginning with anyone of these amino acids. The examiner should note that three groups independently and within three months of each other obtained the gene encoding human tissue factor, determined that the transmembrane region could be deleted and that a truncation could be made at amino acid 219, or shortly thereafter, to yield a soluble protein.

The new claims are drawn to recombinant proteins. Recombinant proteins are inherently distinct from the natural protein when, as in this case, the protein is glycosylated differently when expressed other than in the natural host cells, or not at all, as when expressed in bacteria. The protein is active when expressed in bacteria, for example, as reported in the PCT application by Mt. Sinai Medical School and Yale University, WO 88/09817, cited in applicants' Information Disclosure Statement. A fusion protein is currently being marketed throughout Europe, reportedly by Boehringer Mannheim. Support for the claims are found, for example, at pages 10-11 and 19-22 (recombinant human tissue

factor expressed in non-human host cells), pages 11-12 (fusion proteins), pages 12-13 (deletions including the transmembrane region, of between one and 30 amino acids) and page 15 (deletion or substitution of transmembrane region to yield a soluble form of recombinant human tissue factor), page 19 (post-translational modification), page 15, lines 12-14 (substitution of a cysteine residue), and page 16 (deletion or substitution of N- or O-linked glycosylation residues); see also pages 34-35 and Figure 2.

With response to the argument that the phrase "from amino acid one to less than amino acid 263" is not supported, the Examiner's attention is again drawn to pages 12-30 which specifically describes deletions of from one to thirty amino acids, as well as the discussion at page 15 regarding deletion of the transmembrane region as necessary to yield a soluble water soluble human tissue factor.

The rejection regarding the deletion of the cytoplasmic region simply flies in the face of conventional knowledge. The specification and claims are to be interpreted in view of the skill in the art. One skilled in the art would find it far easier and more sensical to delete the TF encoding region at one point, rather than re-splice together the useless cytoplasmic region with the biologically active region of the tissue factor.

Moreover, Figure 3a shows that a SspI restriction site is conveniently located approximately between the codons encoding amino acids 219 and 220. One skilled in the art would quickly conclude that cleavage with SspI is one possible method for achieving deletion

of the transmembrane region. Since the next SspI site is located around nucleotide 2050, toward the very end of the untranslated 3′, the region encoding the cytoplasmic domain is inherently deleted with the transmembrane region. Therefore, the specification does teach a soluble tissue factor protein having both the transmembrane and cytoplasmic regions deleted.

The law is clear that one need not disclose all that is known to those skilled in the art. Certainly one would be hard pressed to argue why deletion of amino acids 220 to 263 would not be obvious from what is disclosed. The ability of those skilled in the art, particularly the conclusions which would naturally be drawn from reading a restriction map, is **most certainly** relevant to what is disclosed in the specification.

While the publications provided by applicants in support of the breadth of their claims cannot be used to **supplement** their disclosure, they can certainly be used to **demonstrate the truth of the statements made in the specification** (variants can be easily identified and/or formed given the hindsight of the complete sequence disclosed in the application and defined by the claims as amended). This is well established law, *e.g. In re Marzocchi*, 439 F.2d 220, 223, fn. 4, (CCPA 1971).

Allowance of all claims 4-6, 8, and 20-26, as amended, and 27-30, is earnestly solicited.

Respectfully submitted,

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CERTIFICATE OF MAILING UNDER 37 CFR §1.8a

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Date: April 17, 1996

Patrea L. Pabst

Claims Pending:

4. (three times amended) Purified human tissue [Tissue] factor protein selected from the group consisting of tissue factor having an amino acid sequence as provided in Figure 2 from at least amino acid residue three to at least amino acid residue 219, human tissue factor having an amino acid sequence as provided in Figure 2 from at least amino acid residue three to at least amino acid residue 219 [wherein a hydrophilic residue is substituted for a hydrophobic residue, tissue factor wherein a cysteine or proline is substituted with any other amino acid residue, tissue factor wherein a residue having an electropositive side chain is substituted for an electronegative residue,] and human tissue factor having an amino acid sequence as provided in Figure 2 from at least amino acid residue three to at least amino acid residue 219 wherein a residue]having a bulky side chain is substituted for one not having a side chain] at an N- or O-glycosylation site is substituted or deleted, wherein the tissue factor has activity in a clotting assay with human plasma.

5. The tissue factor protein of claim 4 wherein the transmembrane domain is deleted.

6. (twice amended) The tissue factor protein of claim 4 having [the mature tissue factor protein] an amino acid sequence [of] as provided in Figure 2 from at least amino acid residue three to at least amino acid residue 219 [in which from one to ten amino acids have been inserted, deleted or substituted].

8. (twice amended) The tissue factor protein of claim [6] 4 having an amino acid sequence as provided in Figure 2 [wherein the amino acid residues 221 to 241 are deleted].

20. (twice amended) A soluble isolated tissue factor with the amino acid sequence shown in Figure 2 from amino acid one, two or three to an amino acid residue selected from the group between amino acid residues 219 to [less than] amino acid residue 263, wherein the tissue factor has activity in a clotting assay. 21. The tissue factor of claim 20 which is not glycosylated.

22. (amended) The tissue factor of claim 20 consisting of the amino acid residues of Figure 2 from residues one to 219.

23. (amended) The tissue factor of claim 20 having an amino acid sequence of Figure 2 from between amino acid one and between residues 220 and 263.

24. The tissue factor of claim 20 wherein the cysteine residues are substituted with other amino acids.

25. The tissue factor of claim 20 wherein the potential proteolysis sites are deleted by replacing the amino acids with glutaminyl or histidyl residues or deleting one of the basic residues.

26. (amended) The tissue factor of claim 20 having the sequence shown in Figure 2 between amino acid residues one, two or three and 219 and [at least some of the region] between amino acid residues 243 to 263.

27. A recombinant human tissue factor comprising the amino acid sequence shown in Figure 2 from amino acid residue three to amino acid residue 219 expressed in a host cell selected from the group consisting of procaryotic cells, non-human animal cells, insect cells, plant cells, and yeast, having activity in a clotting assay.

28. The recombinant human tissue factor of claim 27 which is not glycosylated.

29. The recombinant human tissue factor of claim 27 comprising the amino acid sequence shown in Figure 2 from amino acid residue three to amino acid residue 263.

30. An recombinant human tissue factor fusion protein having activity in a clotting assay.